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p62 at the Crossroads of Autophagy, Apoptosis, and Cancer

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The signaling adaptor p62 is a multidomain protein implicated in the activation of the transcription factor NF-κB. Recent findings link p62 activity to the extrinsic apoptosis pathway, and Mathew et al. (2009) now show that the modulation of p62 by autophagy is a key factor in tumorigenesis. These findings place p62 at critical decision points that control cell death and survival.

The intricate signaling network that determines whether cells grow, senesce, or die achieves a remarkable degree of specificity with a relatively small number of signaling molecules. This fidelity is possible because of the multidomain protein adapters that organize signaling traffic (Moscat et al., 2007). One such adaptor is p62 (also known as sequestosome-1), which was initially isolated as an interacting partner of atypical protein kinase C (aPKC). Studies employing knockout, transgenic, and knockin mice have shown that p62 plays critical roles in a number of cellular functions, including bone remodeling, obesity, and cancer (Duran et al., 2004, 2008; Moscat et al., 2006; Rodriguez et al., 2006).

In this Minireview, we discuss exciting new observations about p62. Recent work reveals that p62 acts as a signaling hub through its ability to recruit and oligomerize important signaling molecules in cytosolic speckles to control cell survival and apoptosis (Sanz et al., 2000; Jin et al., 2009). In findings reported in this issue, Mathew et al. (2009) show that the elimination of p62 by autophagy suppresses tumorigenesis. These discoveries suggest that p62 is a central player in the life and death decisions of the cell.

p62 Signaling Complexes and Intracellular Speckles

The structure of p62 reveals a rich potential for interacting partners consistent with its role as a focal point in signal transduction (Figures 1A and 1B). Among its many domains, p62 has a PB1 domain, which is a protein-protein interaction module present in other signaling molecules, such as the aPKCs and the polarity protein Par-6. aPKCs and p62 bind each other through their PB1 domains, and this binding is implicated in the activation of the transcription factor NF-κB downstream of cell stimulation by interleukin 1 (IL-1), RANK ligand (RANKL), or nerve growth factor (NGF) (Sanz et al., 2000; Duran et al., 2004). Additionally, the PB1 domain of p62 also facilitates its oligomerization, which is thought to be critical for its cellular function (Moscat et al., 2006, 2007). Notably, the ubiquitin-associated (UBA) domain is also essential for the function of p62 (Moscat et al., 2007).

p62 is easily spotted in cytosolic cellular speckles, or aggregates, formed of PB1-driven p62 oligomers and p62-aPKC complexes, as well as polyubiquitin-conjugated proteins (Sanz et al., 2000; Moscat et al., 2006; Pankiv et al., 2007; Jin et al., 2009). These speckles are signal-organizing centers where p62 interacts with TRAF6 and caspase-8 (Jin et al., 2009; Sanz et al., 2000). TRAF6 is a lysine 63 (K63) E3 ubiquitin ligase involved in NF-κB activation (Moscat et al., 2006). Importantly, the interaction of p62 with TRAF6 promotes its oligomerization and subsequent activation, which leads to K63 polyubiquitination of TRAF6 resulting in the activation of NF-κB (Sanz et al., 2000; Moscat et al., 2006).

p62 mutations are associated with Paget's disease of bone (PDB) (Laurin et al., 2002), a genetic disorder characterized by enhanced osteoclastogenic activity, and mice with altered p62 activity display bone phenotypes consistent with effects on osteoclasts (Duran et al., 2004; Kurihara et al., 2007; Hiruma et al., 2008; Laurin et al., 2002). Interestingly, mice lacking TRAF6, like those lacking p62, are characterized by defective osteoclastogenesis (Moscat et al., 2006). In addition, transgenic and knockin mice expressing the PDB mutation in p62 display increased NF-κB signaling and have a tendency toward enhanced osteoclastogenesis. These findings validate the relevance of a RANK-p62-TRAF6-NF-κB axis to human physiology (Figure 1B) (Hiruma et al., 2008; Kurihara et al., 2007).

The ability of p62 to oligomerize and aggregate serves to catalyze the efficiency of TRAF6 effects, which requires the TRAF6-binding domain of p62 as well as its UBA and PB1 domains (Moscat et al., 2006). Therefore, during IL-1 stimulation p62 recruits a TRAF6-IRAK complex to the cytosolic speckles consisting of small aggregates where polyubiquitin-linked signaling is efficiently activated to achieve NF-κB stimulation (Sanz et al., 2000). These p62-TRAF6 signaling hubs can also be involved in cell-signaling regulation through the modulation of receptor trafficking (Geetha et al., 2005).

Interestingly, the mechanism of action of death receptors DR4 and DR5 also needs p62 for optimal function. Thus, cell activation by TRAIL promotes the polyubiquitination of caspase-8 through a cullin-3-dependent process (Jin et al., 2009). Notably, p62 associates with polyubiquitinated caspase-8, and its recruitment to oligomeric speckles is required to trigger the apoptotic pathway (Jin et al., 2009). This suggests that the p62 speckles are signaling hubs that could determine whether cells survive, by triggering the TRAF6-NF-κB pathway, or die, by activating the aggregation of caspase-8 and its downstream effector caspases.

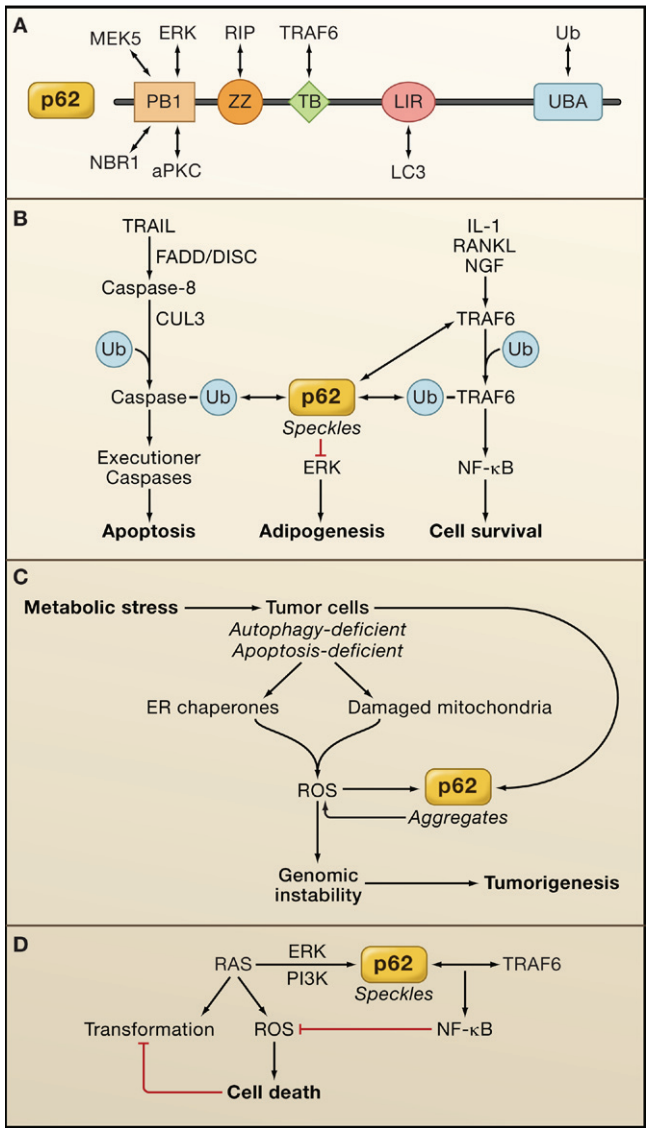

Figure 1. Cellular Functions of p62

(A) Structural domain organization of p62 and p62-interacting partners. p62 has a PB1 domain, a ZZ-type zinc finger domain, a TRAF6-binding (TB) domain, an LC3-interacting region (LIR), and a ubiquitin-associated domain (UBA).

(B) p62 speckles promote the oligomerization of TRAF6 and caspase-8 leading to the enhancement of NF-κB activation and apoptosis, respectively. p62 speckles also sequester active ERK, restraining adipogenesis.

(C) Autophagy deficiency in apoptosis-impaired tumor cells leads to increased p62 aggregation. This triggers a positive feedback loop for the generation of reactive oxygen species (ROS) and enhanced genomic instability and tumorigenesis.

(D) Ras-induced transformation promotes the accumulation of p62, which leads to an increase in NF-κB activity, thereby restraining ROS production and inhibiting tumor cell death.

DISC, death-inducing signaling complex; ER, endoplasmic reticulum.

Control of p62 by Autophagy

The fact that p62 binds the autophagy regulator Atg8/LC3 through a region termed the LC3-interacting region (LIR) (Figure 1A) combined with the observation that p62 accumulates in autophagy-deficient mice suggests a link between autophagy and p62 (Komatsu et al., 2007). p62 along with its PB1 partner, NBR1 (neighbor of BRCA1 gene 1), have been proposed to regulate the packing and delivery of polyubiquitinated, misfolded, aggregated proteins and dysfunctional organelles for their clearance through autophagy in mammalian cells and in the fruit fly *Drosophila* (Kirkin et al., 2009; Nezis et al., 2008; Kim et al., 2008; Pankiv et al., 2007). This new role for p62 could also regulate cell survival, as the disposal of toxic aggregates is essential for the prevention of cell death in several pathological situations in which p62 is a component of these aggregates (Zatloukal et al., 2002). Mice lacking Atg7 are impaired in autophagy, leading to the appearance in the brain and liver of polyubiquitinated aggregates that colocalize with p62 (Komatsu et al., 2007). In mice lacking both Atg7 and p62, these aggregates are completely absent, suggesting that p62 plays a structural role in their formation (Komatsu et al., 2007). This indicates that p62, in addition to its role as a signaling hub whose levels can be regulated by autophagy, may also be a central element in a quality-control mechanism for the disposal of toxic aggregates. However, this model presents a number of apparent inconsistencies that need to be resolved.

First, recent data demonstrate that the proteasome is also inhibited in autophagy-deficient cells due to the accumulation of p62 (Korolchuk et al., 2009). This might reduce the degradation of polyubiquitinated proteins, resulting in the appearance of polyubiquitinated aggregates—not as a consequence of a structural role for p62 but because of impaired proteasome activity (Korolchuk et al., 2009). However, if p62 expression is ablated in autophagy-deficient cells, the proteasome is not inhibited, the accumulated polyubiquitinated proteins are efficiently degraded, and aggregates do not form (Korolchuk et al., 2009). This might explain the lack of aggregates in the mice lacking both Atg7 and p62 without invoking a role for p62 in building up the aggregates (at least in liver, in which the accumulation of polyubiquitinated proteins in the double knockout mice is lower than that found in mice deficient in only Atg7) (Komatsu et al., 2007). The second problem with p62 being a "garbage disposer" of toxic aggregates is the nature of those aggregates. It is believed that by forming these aggregates p62 concentrates toxic misfolded proteins for two purposes: (1) to remove them from the soluble fraction, thus reducing their toxicity and (2) to direct these "packaged" aggregates to the degradative route. If the role of p62 is to remove these "toxic assets," then more liver damage would be expected in mice deficient in both Atg7 and p62 than in mice only deficient in Atg7. Surprisingly, however, the loss of p62 lessens the liver damage caused by deficient autophagy in *Atg7* knockout mice (Komatsu et al., 2007). One possible explanation for these findings is based on the role of p62 in activating the caspase pathways (Jin et al., 2009). If liver toxicity associated with impaired autophagy leads to caspase activation and apoptosis, the loss of p62 in Atg7-deficient livers could dampen caspase-activated apoptosis. This could explain why the livers of *Atg7/p62* double knockout mice are healthier than those in mice deficient in Atg7 alone. Detailed biochemical analysis of livers from mice lacking both Atg7 and p62 should shed light on this question. In any case, clarifying the nature and composition of these aggregates and their relation to the p62 signaling speckles detected in cells activated by IL-1 or TRAIL is necessary for a better

understanding of the quality-control mechanisms that go awry in cells with impaired autophagy or proteasome function. Such studies would lead to a better understanding of the pathological situations characterized by the formation of p62-containing protein aggregates (Zatloukal et al., 2002).

p62, a Critical New Player in Cancer

The findings of Mathew et al. (2009) provide evidence that impaired autophagy leads to p62 accumulation, thereby promoting tumorigenesis. This study grew from the paradoxical observation that autophagy is generally considered a survival pathway in cells undergoing nutrient deprivation; however, allelic loss of the autophagy gene *beclin1* occurs in human tumors and favors carcinogenesis in mice (Mathew et al., 2007). In prior work, White and coworkers have suggested that a potential explanation for this conundrum is that, under conditions of aggressive tumorigenesis associated with metabolic stress, autophagy may prevent genome instability thereby restraining tumorigenesis (Mathew et al., 2007). Importantly, in autophagy-deficient and apoptosis-incompetent tumor cells, metabolic stress leads to the accumulation of p62, elevated expression of endoplasmic reticulum (ER) chaperones, damaged mitochondria, and reactive oxygen species (ROS) (Mathew et al., 2009). This increase in p62 levels is critical for tumorigenesis, as overexpression of p62 in their cell model leads to increased tumor volume in mouse xenograft experiments (Mathew et al., 2009) (Figure 1C). These results are consistent with previous reports demonstrating that p62 is required for Ras-induced tumorigenesis in vitro and in vivo (Duran et al., 2008). In these studies, it is shown that Ras-induced transformation is impaired in immortalized embryo fibroblasts from p62-deficient mice due to a decrease in NF-κB activation (Figure 1D). Interestingly, p62 transcription is induced in the Ras transformants through a mechanism that involves ERK and PI-3 kinase, two bona fide downstream targets of Ras, and the AP1 element in the *p62* promoter (Duran et al., 2008). The reduced NF-κB activation observed in the p62-deficient cells leads to lower levels of ROS scavengers, which results in enhanced ROS levels and more apoptosis. This could explain the reduced carcinogenic potential of p62-deficient cells (Duran et al., 2008). These cell-culture studies are validated in a mouse model of cancer that closely mimics human lung adenocarcinoma. In these mice, oncogenic Ras is inducibly expressed in type II alveolar epithelial cells. Consistent with the data from embryo fibroblasts, the lack of p62 completely abrogates Ras-induced lung adenocarcinomas and, importantly, inhibits the Ras-induced nuclear translocation of NF-κB in the lung cells in vivo (Duran et al., 2008). The mechanism whereby p62 channels Ras signals to NF-κB involves the activation of TRAF6 (Figure 1D). This suggests that Ras, by inducing the expression of p62, utilizes a pathway designed for the activation of NF-κB by RANK and IL-1 signaling to increase the survival of tumor cells by reducing damage due to Ras-activated ROS (Duran et al., 2008). These results, combined with observations that p62 levels are increased in at least 60% of human lung adenocarcinomas and in 90% of human lung squamous cell carcinomas (M.T.D.-M. and J.M., unpublished data), support a role for p62 in lung tumorigenesis.

The recent report of Mathew et al. adds a number of interesting twists to the role of p62 in cancer. These investigators show that when cells with impairment in both apoptosis and autophagy are metabolically stressed, the accumulation of p62 leads to enhanced tumorigenicity through an as yet undefined mechanism involving increased aneuploidy (Mathew et al., 2009). These observations place p62 as the missing link between deficient autophagy and increased tumorigenesis through the control of genome instability (Mathew et al., 2007). An interesting feed-forward loop may contribute to this process. The increased ROS production in these cells might be responsible, at least in part, for the induction of p62 expression. p62 overexpression then contributes to additional ROS production as part of an amplifying loop, thereby promoting genome instability (Figure 1C) (Mathew et al., 2009).

A puzzling aspect of these studies is that the overexpression of p62 correlates with reduced NF-κB activation (Mathew et al., 2009). In contrast, other work has shown that activation of NF-κB is a consequence of p62 overexpression or activating PDB mutations in vitro and in vivo (Duran et al., 2004; Kurihara et al., 2007). Moreover, a lack of p62 reduces NF-κB stimulation in Ras-transformed embryo fibroblasts and in the mouse model of Ras-induced lung adenocarcinoma (Duran et al., 2008). Also, in the lung and fibroblast models, the lack of p62 leads to increased ROS due to inhibited NF-κB (Duran et al., 2008) (Figure 1D), whereas the opposite is found in the immortalized baby mouse kidney (iBMK) cell system utilized by Mathew et al. The reasons for this apparent discrepancy are unclear. One important difference is that the Ras-transformed cells were cultured with plentiful nutrients and with the autophagy and apoptosis pathways intact (Duran et al., 2008). It is plausible that when cells are deprived of nutrients and oxygen, and p62 overexpression reaches a certain threshold level, NF-κB is inhibited by the mopping up of critical components of the NF-κB pathway into huge p62 aggregates. These stand in contrast to the smaller p62-positive speckles (Sanz et al., 2000), which activate NF-κB. A similar scenario has been proposed to explain how p62 sequesters and inhibits the kinase ERK, which has repercussions for the roles of both proteins in adipogenesis and obesity (Rodriguez et al., 2006). The surprising inhibitory effect of p62 on NF-κB might also be related to the fact that the lack of autophagy does not result in the accumulation of polyubiquitinated proteins in the modified iBMK tumor system (Mathew et al., 2009), whereas polyubiquitinated proteins do accumulate in other in vivo systems (Komatsu et al., 2007). It is possible that iBMK tumor cells have evolved mechanisms to prevent increased polyubiquitination that, in the case of p62 and TRAF6, has been shown to be essential for NF-κB activation.

The cell system utilized by Mathew et al. is valuable because it could mimic a tumor that is experiencing nutrient and oxygen deprivation, and in which the apoptosis and autophagy pathways are inhibited. However, it is clear that the loss of p62 correlates with decreased NF-κB activation in pneumocytes expressing oncogenic Ras (Duran et al., 2008). This is true for embryo fibroblasts and osteoclasts as well, strongly supporting a role for p62 as a physiologically relevant activa-

tor of NF-κB. Further studies should clarify how p62 switches from an activator of NF-κB to an inhibitor under conditions of metabolic stress.

The fact that the modified iBMK cells have blunted apoptosis also precludes a test of the role of p62 in caspase activation in cancer, as demonstrated by Jin et al. (2009). It is possible that in tumor cells with intact apoptosis pathways, p62 serves to fine-tune the balance between antiapoptotic and proapoptotic signals. This subtle homeostatic process might be completely subverted when autophagy genes are lost or when autophagy is inhibited as a consequence of hyperactivation of the PTEN/Akt/TOR pathway. Another example would be organs, such as the liver, in which compensatory proliferation in the presence of an inflammatory response might convert a tumor-promoting signal, like NF-κB, into a tumor-suppressor pathway. The role of p62 in the disposal of damaged organelles and misfolded proteins in aberrantly growing tumor cells are additional questions to consider.

The exciting recent observations establish p62 as a potential therapeutic target in at least some types of cancer. These findings also expose the need for further investigation concerning the role of p62 in tumorigenesis, given that different gene mutations, cell types, and environmental conditions appear to dramatically influence its effects.

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REFERENCES

Duran, A., Serrano, M., Leitges, M., Flores, J.M., Picard, S., Brown, J.P., Moscat, J., and Diaz-Meco, M.T. (2004). Dev. Cell *6*, 303–309.

Duran, A., Linares, J.F., Galvez, A.S., Wikenheiser, K., Flores, J.M., Diaz-Meco, M.T., and Moscat, J. (2008). Cancer Cell *13*, 343–354.

Geetha, T., Jiang, J., and Wooten, M.W. (2005). Mol. Cell *20*, 301–312.

Hiruma, Y., Kurihara, N., Subler, M.A., Zhou, H., Boykin, C.S., Zhang, H., Ishizuka, S., Dempster, D.W., Roodman, G.D., and Windle, J.J. (2008). Hum. Mol. Genet. *17*, 3708–3719.

Jin, Z., Li, Y., Pitti, R., Lawrence, D., Pham, V., Lill, J., and Ashkenazi, A. (2009). Cell *137*, 721–735.

Kim, P.K., Hailey, D.W., Mullen, R.T., and Lippincott-Schwartz, J. (2008). Proc. Natl. Acad. Sci. USA *105*, 20567–20574.

Kirkin, V., Lamark, T., Sou, Y.S., Bjorkoy, G., Nunn, J.L., Bruun, J.A., Shvets, E., McEwan, D.G., Clausen, T.H., Wild, P., et al. (2009). Mol. Cell *33*, 505–516.

Komatsu, M., Waguri, S., Koike, M., Sou, Y.S., Ueno, T., Hara, T., Mizushima, N., Iwata, J., Ezaki, J., Murata, S., et al. (2007). Cell *131*, 1149–1163.

Korolchuk, V.I., Mansilla, A., Menzies, F.M., and Rubinsztein, D.C. (2009). Mol. Cell *33*, 517–527.

Kurihara, N., Hiruma, Y., Zhou, H., Subler, M.A., Dempster, D.W., Singer, F.R., Reddy, S.V., Gruber, H.E., Windle, J.J., and Roodman, G.D. (2007). J. Clin. Invest. *117*, 133–142.

Laurin, N., Brown, J.P., Morissette, J., and Raymond, V. (2002). Am. J. Hum. Genet. *70*, 1582–1588.

Mathew, R., Kongara, S., Beaudoin, B., Karp, C.M., Bray, K., Degenhardt, K., Chen, G., Jin, S., and White, E. (2007). Genes Dev. *21*, 1367–1381.

Mathew, R., Karp, C., Beaudoin, B., Vuong, N., Chen, G., Chen, H., Bray, K., Reddy, A., Bhanot, G., Gelinas, C., et al. (2009). Cell, this issue.

Moscat, J., Diaz-Meco, M.T., Albert, A., and Campuzano, S. (2006). Mol. Cell *23*, 631–640.

Moscat, J., Diaz-Meco, M.T., and Wooten, M.W. (2007). Trends Biochem. Sci. *32*, 95–100.

Nezis, I.P., Simonsen, A., Sagona, A.P., Finley, K., Gaumer, S., Contamine, D., Rusten, T.E., Stenmark, H., and Brech, A. (2008). J. Cell Biol. *180*, 1065–1071.

Pankiv, S., Clausen, T.H., Lamark, T., Brech, A., Bruun, J.A., Outzen, H., Overvatn, A., Bjorkoy, G., and Johansen, T. (2007). J. Biol. Chem. *282*, 24131–24145.

Rodriguez, A., Duran, A., Selloum, M., Champy, M.F., Diez-Guerra, F.J., Flores, J.M., Serrano, M., Auwerx, J., Diaz-Meco, M.T., and Moscat, J. (2006). Cell Metab. *3*, 211–222.

Sanz, L., Diaz-Meco, M.T., Nakano, H., and Moscat, J. (2000). EMBO J. *19*, 1576–1586.

Zatloukal, K., Stumptner, C., Fuchsbichler, A., Heid, H., Schnoelzer, M., Kenner, L., Kleinert, R., Prinz, M., Aguzzi, A., and Denk, H. (2002). Am. J. Pathol. *160*, 255–263.